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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

APPEAL BRIEF FOR THE APPELLANT

Ex parte FREYSSINET et al.

METHOD FOR THE DETERMINATION OF THE PRETHROMBOTIC STATE

Serial No. 09/588,553
Appeal No.:
Group Art Unit: 1641
Examiner: J. Grun

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Respectfully submitted,

Monica Chin Kitts
Attorney for Appellants
Reg. No. 36,105

Attorney Docket No.: 101614-00003
ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
1050 Connecticut Avenue, N.W.,
Suite 400
Washington, D.C. 20036-5339
Tel: (202) 857-6000
Fax: (202) 638-4810
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IV. STATUS OF CLAIMS

The rejection of claims 36-44, 64 and 65, all of the claims under consideration in the present application, is being appealed. Claims 45-63 have been canceled in view of a restriction requirement. The claims on appeal are set forth in the attached Appendix I. Claim 36 is independent with claims 37-44 and 64-65 depending directly or indirectly from claim 36. No claims are allowed.

V. STATUS OF THE AMENDMENTS

The advisory action mailed on March 13, 2002 indicates that the amendments made in the response after final rejection filed on February 28, 2002 will be entered upon filing of a Notice of Appeal and an Appeal Brief.

VI. SUMMARY OF THE INVENTION

The present invention relates to a method for determining the prethrombotic state of an individual. In the present invention, circulating microparticles (which are shed from the surface of activated platelets), stimulated procoagulant cells (e.g. activated platelets) and mixtures thereof are determined in a body fluid sample from an individual

as an indication of the individual's prethrombotic state. In the present invention, the body fluid sample is mixed with a purified receptor specific for a phospholipid to form a complex of the purified receptor and the circulating microparticles and/or procoagulant cells. The purified receptor is bound to a solid phase to facilitate the removal of unbound components thereby eliminating high background signals. The binding of the circulating microparticles and stimulated procoagulant cells to the solid phase via the receptor in the present invention, does not induce unwanted cell activation which would lead to erroneous results. Any complex bound to the solid phase is determined as an indication of an individual's prethrombotic state. The present inventors have found that various diseases can be correlated with the amount of circulating microparticles and stimulated procoagulant cells in an individual's blood as shown in table 1 in the present application. The individual's prethrombotic state is compared to prethrombotic values associated with various diseases and risk factors as in table 1.

VII. ISSUES ON APPEAL

The first issue on appeal is whether the invention claimed in claims 36-44 and 64-65 is enabled by the present invention under 35 USC §112, first paragraph.

The second issue on appeal is whether the invention claimed in claims 36-38, 41, 64 and 65 can reasonably be found obvious under 35 USC §103 over Abrams in view of Rote and Margel, Carriere, or Hajek and/or Harlow.

The third issue on appeal is whether the invention claimed in claims 39-40 can

reasonably be found obvious under 35 USC §103 over Abrams in view of Rote and Margel, Carriere, or Hajek and/or Harlow further in view of Dachary-Prigent.

VIII. GROUPING OF CLAIMS

Each claim of this patent application is separately patentable, and upon issuance of a patent, will be entitled to a separate presumption of validity under 35 U.S.C. § 282. For convenience in the handling of this appeal, claims 36-44, 64 and 65 stand together as one group.

IX. THE FINAL REJECTION

Claims 36-44, 64 and 65 are under consideration and pending in this application. Claims 45-63 have been canceled. No claim stands allowed.

Claims 36-44 and 64-65 were rejected as indefinite under 35 USC §112, second paragraph. The advisory action mailed on March 13, 2002 indicates that the amendments filed on February 28, 2002 obviate this rejection.

Claims 36-44 and 64-65 were finally rejected as lacking enablement under 35 USC §112, first paragraph.

Claims 36-38, 41, 64 and 65 were finally rejected as obvious under 35 USC §103 over Abrams in view of Rote and Margel.

Claims 36-38, 41, 64 and 65 were finally rejected as obvious under 35 USC

§103 over Abrams in view of Rote and Carriere.

Claims 36-38, 41, 64 and 65 were finally rejected as obvious under 35 USC §103 over Abrams in view of Rote and further in view of Hajek and/or Harlow.

Claims 39-40 were finally rejected as obvious under 35 USC §103 over Abrams in view of Rote and Margel further in view of Dachary-Prigent.

Claims 39-40 were finally rejected as obvious under 35 USC §103 over Abrams in view of Rote and Carriere further in view of Dachary-Prigent.

Claims 39-40 were finally rejected as obvious under 35 USC §103 over Abrams in view of Rote and Hajek and/or Harlow further in view of Dachary-Prigent.

X. APPELLANT'S ARGUMENTS AGAINST THE REJECTION OF CLAIMS 36-44 AND 64-65 AS LACKING ENABLEMENT UNDER 35 USC §112, FIRST PARAGRAPH.

The first issue on appeal is whether claims 36-44 and 64-65 lack an enabling description of how a prethrombotic state is compared with prethrombotic values associated with various diseases and risk factors. The Office Actions argue that there is no description of how one performs the comparison or whether any particular level of microparticles and/or stimulated procoagulant cells is associated with a particular disease or risk factor. Applicants respectfully disagree and point out table 1 on page 30 of the present application, which shows the prothrombinase activity associated with various diseases and risk factors. In table 1, the captured activated cells or fragments

(i.e. circulating microparticles and/or stimulated procoagulant cells) were detected using a prothrombinase assay (which detects procoagulant phospholipids on the cells) as described on pages 26-27 of the specification. In addition, example 6 in the present application uses the present invention to assess the thrombotic risk associated with Paroxysmal Nocturnal Hemoglobinuria (PNH). Figure 10 shows the results of detecting activated cells and/or microparticles in patients with PNH, aplastic anemia and healthy controls. Figure 10 and table 1 clearly show that prothrobinase activity, as determined by detecting microparticles and/or stimulated procoagulant cells, is higher in patients with diseases associated with an increased thrombotic risk. Table 1 shows numerous diseases and the prothrombinase activity values associated with the diseases In addition, one skilled in the art could easily determine values for other diseases by using the present invention to compare disease patient values and control values. In view of the fact that example 6 provides a specific example of how a comparison is done to assess the thrombotic risk associated with a particular disease and table 1 shows prothrombinase activity values associated with various diseases, applicants contend that the present application provides an enabling description of how a prethrombotic state is compared with prethrombotic values associated with various diseases and risk factors and request that this rejection be reversed.

XI. APPELLANT'S ARGUMENTS AGAINST THE REJECTION OF CLAIMS 36-38, 41, 64 AND 65 AS OBVIOUS UNDER 35 USC §103 OVER ABRAMS IN VIEW OF ROTE AND MARGEL, CARRIERE, OR HAJEK AND/OR HARLOW.

The second issue on appeal is whether claims 36-38, 41, 64 and 65 can reasonably be found obvious over Abrams in view of Rote and either Margel, Carriere, or Hajek and/or Harlow.

Abrams discloses a method for detecting activated platelets. However, Abrams does not suggest or disclose the use of receptors for phospholipids or phospholipid complexes. As stated on the bottom of page 5 of the Office Action dated February 26, 2002, Abrams "does not teach antibodies for the detection of phosphatidylserine in activated platelet membranes, use of a solid phase such as particulate labels, nor alternatives to flow cytometry". Abrams uses antibodies to surface antigens and flow cytometry for the detection of the activated platelets. In his method, no immobilization and thus no separation of a solid phase (carrying immobilized cells) and a liquid phase is carried out. Therefore Abrams would have a high background signal which is avoided by the present invention. Rote and Margel do not cure the deficiencies in Abrams as neither Rote or Margel suggest or disclose immobilizing the microparticles or cells on a solid phase. Rote also uses flow cytometric analysis to determine the reactivity of his antibodies against platelets and Margel discloses only labels for cell labeling. Though Carriere discloses solid phase immobilization and separation, he does not suggest that such a method can be used to detect stimulated procoagulant cells. Neither Hajek nor Harlow suggest that in vivo activated platelets can be accurately detected using a solid phase method. Therefore, these references do not cure the deficiencies in the other cited references as discussed above.

Applicants respectfully point out that the only references cited above for the detection of activated platelets (Abrams and Rote) use flow cytometry. One of these references, Abrams indicates on page 469, top of the right column, that sample washing or centrifugation may traumatize platelets and induce unwanted cell activation. Therefore, one would not combine Abrams and Rote with Carriere's solid phase method as one would not expect to accurately detect in vivo activated procoagulant cells due to in vitro cell activation caused by the solid phase separation. Applicants respectfully contend that Abrams teaches away from an assay with a solid phase immobilization by stating that "the use of directly conjugated antibodies eliminates the need for addition of secondary antibodies and for sample washing or centrifugation, maneuvers that are likely to traumatize platelets and induce unwanted cell activation in vitro" (page 469, top of right column). In addition, Rote states on page 196, right column, end of the middle paragraph, that 'occasional reactivity was observed against "unactivated" platelets that had been partially activated in response to trauma during the preparation procedure'. In view of the fact that the only references cited for the detection of activated platelets (Abrams and Rote) use flow cytometry and indicate that unactivated platelets can be partially activated due to handling procedures, applicants contend that one skilled in the art would not combine these references with a solid phase assay to arrive at the present invention.

The present invention makes it possible to detect a thrombotic risk or to detect a high level of cell activation (e.g. apotosis) in body fluids by taking into account the

properties of phospholipids or phospholipid complexes that are exposed on circulating microparticles or cells. The present invention uses an immobilization step on a solid phase followed by the separation of solid phase (carrying microparticles or cells) and liquid phase. This detection system permits the measurement of the amount of immobilized phospholipids based on the real in vivo procoagulant potential of the microparticles or cells that possess exposed phospholipids. The whole detection process takes place on the biological cell membrane from an activated human cell.

The capture of the activated cells or fragments thereof on a solid phase is essential to allow the separating steps to be performed. Using these steps, a high background signal is eliminated. By using antibody-dependent immobilization of cells or microparticles the present system permits the measurement of the procoagulant potential linked to a specific type of cells or microparticles that are characterized and captured through a specific membrane linked antigen.

Since neither Abrams, Rote, Margel, Carriere, or Hajek and/or Harlow individually or in combination suggest or disclose immobilization and separation of a solid phase carrying immobilized activated cells, the combination of these references does not suggest a method to detect a thrombotic risk which takes into account the properties of phospholipids exposed on microparticles, without a high background signal, as in the present invention. In view of the above discussion, applicants request that this rejection be reversed.

XII. APPELLANT'S ARGUMENTS AGAINST THE REJECTION OF CLAIMS 39-40 UNDER 35 USC §103 OVER ABRAMS IN VIEW OF ROTE AND MARGEL, CARRIERE, OR HAJEK AND/OR HARLOW FURTHER IN VIEW OF DACHARY-PRIGENT

The third issue on appeal is whether claims 39-40 can reasonably be found obvious over Abrams in view of Rote and Margel, Carriere, or Hajek and/or Harlow further in view of Dachary-Prigent. As discussed above, Abrams and Rote disclose the detection of activated platelets and both of these references use flow cytometry to detect the platelets. These references also indicate that inactivated platelets can become partially activated by preparation procedures such as washing and centrifugation. In view of this, one skilled in the art would not be motivated to combine Abrams and Rote with a solid phase assay as disclosed in Carriere or an agglutination assay as disclosed in Hajek and Harlow as such a procedure would be expected to traumatize and partially activate the cells producing a false positive result. Margel discloses only cell labels and does not suggest that unactivated platelets can be immobilized on a solid phase without activating the cells. Dachary-Prigent teaches the use of annexin V as a probe for phospholipids in the presence of calcium and the use of flow cytometry to assess activated platelets. Dachary –Prigent does not suggest or disclose that unactivated platelets can be immobilized on a solid phase without activating the cells and thus does not cure the deficiencies in the other cited art as discussed above. Applicants point out that all three of the references which detect activated platelets use flow cytometry despite the fact that solid phase assays were known in the art at the time these references were published. Applicants contend that

this is because it was known that certain handling procedures such as washing and centrifugation, can activate platelets thereby producing erroneous results. Flow cytometry was chosen as the detection method to avoid unwanted activation. The present inventors have surprisingly found that activated procoagulant cells and microparticles can be determined using a solid phase assay without producing unwanted activation. In view of the above discussion, applicants contend that this rejection should be reversed.

For all of the above noted reasons, it is strongly contended that certain clear differences exist between the present invention as claimed in claims 36-44 and 64-65 and the prior art relied upon by the Examiner. It is further contended that these differences are more than sufficient evidence that the present invention would not have been obvious to a person having ordinary skill in the art at the time the invention was made.

This final rejection being in error, therefore, it is respectfully requested that this honorable Board of Patent Appeals and Interferences reverse the Examiner's decision in this case and indicate the allowability of application claims 36-44 and 64-65

In the event that this paper is not being timely filed, the applicant respectfully petitions for an appropriate extension of time. Any fees for such an extension together with any additional fees which may be due with respect to this paper may be charged to counsel's deposit account 01-2300.

Respectfully submitted,

Monica Chin Kitts
Attorney for Applicants
Registration No. 36,105

Customer No. 004372
ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
1050 Connecticut Avenue, N.W., Suite 400
Washington, D.C. 20036-5339
Tel: (202) 857-6000
Fax: (202) 638-4810

APPENDIX 1

CLAIMS ON APPEAL

36. A method for determining a prethrombotic state of an individual for screening for disease and identification of risk factors, comprising:

obtaining a body fluid sample comprising a member selected from the group consisting of circulating microparticles, stimulated procoagulant cells and mixtures thereof;

mixing the sample containing said member with a purified receptor specific for a phospholipid, under conditions to form a complex of the purified receptor and said member, wherein said purified receptor is bound to a solid phase,

removing unbound components;

determining any complex bound to said solid phase; as an indication of an individual's prethrombotic state; and

comparing the individual's prethrombotic state to prethrombotic values associated with an assortment of diseases and risk factors, thereby screening for disease and identifying risk factors.

37. The method according to claim 36, wherein said complex is determined directly on said solid phase.

38. The method according to claim 36, wherein said complex is determined after

removing said complex from said solid phase.

39. The method according to claim 36, wherein said purified receptor is annexin V.

40. The method according to claim 39, further comprising adding calcium ions in step A.

41. The method according to claim 36, wherein said purified receptor is bound to the solid phase via a specific binding pair comprising a first and a second binding pair member, and wherein said first binding pair member is attached to the solid phase and said second binding pair member is coupled to said purified receptor.

42. The method according to claim 36, wherein in step (c) the complex is determined by detecting any activation of prothrombin (factor II) to thrombin (factor IIa)

43. The method according to claim 42, wherein inhibitors of thrombin, Factor Xa or both thrombin and factor Xa are present during step (a).

44. The method according to claim 42, wherein the activation of prothrombin to thrombin is detected by reacting the complex with a reagent comprising factor V, factor Xa,

prothrombin (factor II), and calcium ions, stopping the reaction by complexation of the calcium ions, and determining thrombin by its ability to hydrolyze a chromogenic substate.

64. The method of claim 36, wherein said purified receptor is bound directly to the solid phase.

65. The method according to claim 36, wherein said purified receptor is bound indirectly to a solid phase.